Using Microwells for Controlled Intestinal Differentiation of hiPS Cells

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Abstract

Recent advancements in developmental biology and tissue engineering have greatly contributed to pharmaceuticals and drug testing by allowing for the development of more efficient and accurate *in vitro* models of human tissues. In particular, the Wells lab, at the Cincinnati Children's Hospital Medical Center, has developed a protocol for the differentiation of human induced pluripotent stem cells (hiPS cells) into intestinal organoids. However, this protocol, which mimics the *in vivo* microenvironment of developing definitive endoderm and mid-hindgut, often results in the random generation of gut spheroids, which later mature into organoids. The complex development of hindgut does not allow for finer analysis of the local environment pertinent to the development of an individual spheroid. To address this problem, we employed hydrogel-based microwells. By attempting to differentiate hiPS cells into mid-hindgut tissue in microwells of varying 2D dimensions, depth, and spacing, we were able to deduce more optimal conditions for the *in vitro* development of individual spheroids. This method will allow for improved analysis of the local factors important for spheroid generation because it reduces the previously complex culture over a relatively large surface area to the smallest-budding unit. Using this approach to gain a better understanding of the local environment may lead to an improved differentiation protocol that will result in more organized intestinal tissue useful for drug testing and disease modelling.

Background: Intestinal Differentiation

Previously, the Wells lab has developed a protocol to differentiate human induced pluripotent stem cells into intestinal organoids *in vitro*[1].

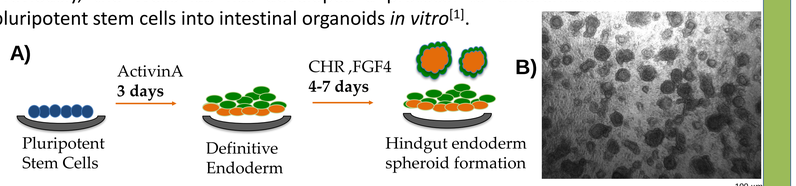


Figure 1 hiPS cells to hingut differentiation. **A)** ActivinA is first used to differentiate hiPS cells into definitive endoderm. Then, the cells are subjected to FGF4 and CHIR99021 to be further differentiated into hindgut and generate spheroids, which later mature into organoids (after being embedded in matrigel). **B)** Bright-field image of spheroids taken on day 9 of differentiation. 5X

Background: Microwells

PEG-based microwells have been used to culture stem cells in an effort to produce homogeneous embryoid bodies[2]. Here, we make microwells of varying dimensions using an ink template to pattern UV-induced polymerization of PEGDA.

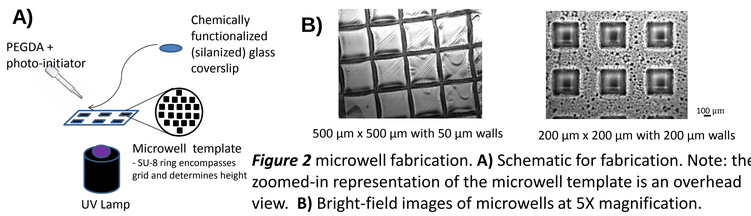


Figure 2 microwell fabrication. **A)** Schematic for fabrication. Note: the zoomed-in representation of the microwell template is an overhead view. **B)** Bright-field images of microwells at 5X magnification.

Objective and Motivation

The current protocol for intestinal differentiation of hiPS cells is complex and produces hundreds of spheroids over 2 cm^2, making it difficult to identify key components for spheroid budding in the microenvironment. Our goal is to use microwells to culture the smallest-budding unit of intestinal spheroids to better analyze the microenvironment pertinent to spheroid formation and to model the budding process for more reproducible and controllable differentiation.

Three Experimental Challenges

1) Differentiating hiPS cells on a glass surface.
- Has not been done before
- Standard procedure requires plastic surface

2) Maintaining hiPS cells' pluripotency in microwells.
- Pluripotency is a stem cell's ability to differentiate into any cell type in the body

3) Differentiating hiPS cells into hindgut in microwells
- That is, generating spheroids (eventually finding the smallest-budding unit)

1) Can hiPS cells adhere to and be differentiatied on a silanized glass surface?
- Cultured hiPS cells on a silanized glass surface for 4 days.
- Fixed cells and stained for pluripotency markers Sox2 and Oct4 (Fig. 3A).
- Compared the differentiation of hiPS cells on plastic and glass surfaces (Fig. 3B) and stained for definitive endoderm markers after 4 days.

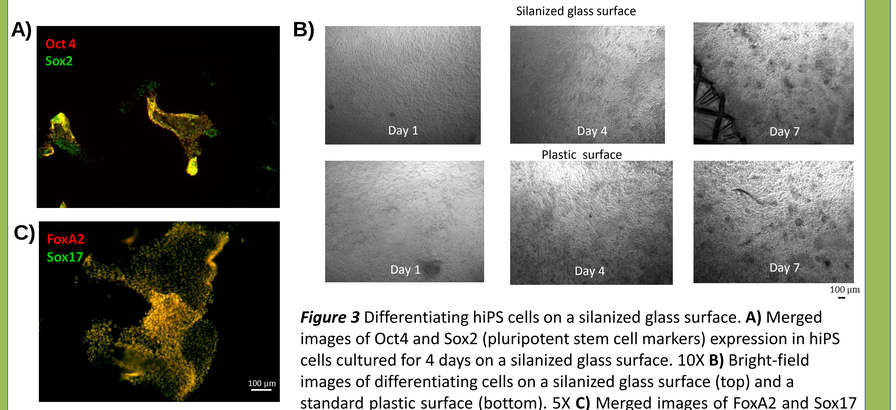


Figure 3 Differentiating hiPS cells on a silanized glass surface. **A)** Merged images of Oct4 and Sox2 (pluripotent stem cell markers) expression in hiPS cells cultured for 4 days on a silanized glass surface. 10X **B)** Bright-field images of differentiating cells on a silanized glass surface (top) and a standard plastic surface (bottom). 5X **C)** Merged images of FoxA2 and Sox17 (definitive endoderm markers) expression on 4th day of differentiation on glass surface. 10X

2) Can hiPS cells remain pluripotent in microwells?
- HiPS cells were seeded onto matrigel-coated microwells of various dimensions
- After 4 days, the cells were fixed and stained for Sox2 and Oct4 (Fig. 4A).

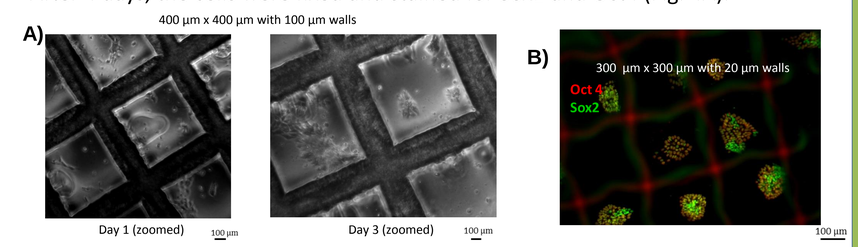
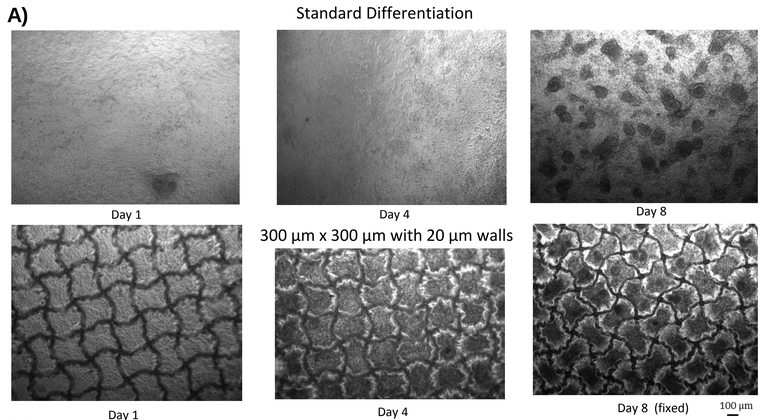


Figure 4 Maintenance of hiPS cells' pluripotency in microwells. **A)** Bright-field images. 5X, zoom **B)** Merged images for Oct4 and Sox2 (pluripotent stem cell markers) expression. 10X

Results

3) Evaluating success of differentiation protocol in mircowells.
- Compared differentiating cells in microwells with differentiating cells on a standard plastic surface (Fig. 5A).
- Stained for definitive endoderm (FoxA2 and Sox17) and hindgut markers (Cdx2 and Sox2) 4 and 8 days into differentiation, respectively (Fig. 5B) [3][4], note the improved expression in the large break.



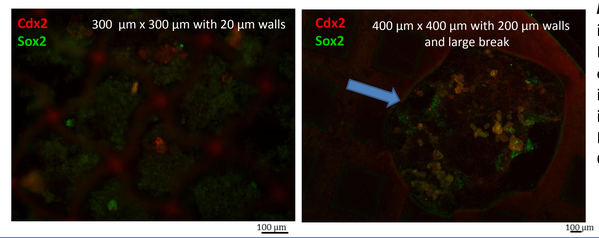


Figure 5 Differntiation in microwells. **A)** Bright-field images of standard (top) and in-microwell different-iation (bottom). 5X **B)** Merged images of Cdx2 and Sox2 (hind-gut and foregut markers, respectively)

Conclusion

- hiPS cells can remain pluripotent in microwells and are likely able to be differentiated into definitive endoderm and (subsequently) into hindgut in microwells.
- Larger wells and thinner walls have been more effective for maintenance of pluripotency and for differentiation. Could the PEGDA be absorbing larger amounts of growth factors and intercellular signals in microwells with thicker walls?

Future

1) Examine effects of wall thickness/depth and combine different dimensions and wall sizes on one grid.
2) Optimize microwells to culture smallest-budding unit for intestinal spheroids.
3) Analyze the microenvironment pertinent to spheroid generation.
4) Model budding process for more reproducible and controllable differentiation.

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